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Assessing Safety of Thrombolytic Therapy

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Abstract

Thrombolytic therapy involves thrombolytic agents administered to patients suffering from venous or arterial thrombosis. The therapy induces systemic effects interrelated with the thrombolytic agent used. Bleeding is a prominent complication of thrombolytic therapy. Exhaustion of coagulation factors, generation of excessive amounts of fibrin degradation products (FDPs), therapy-induced activation of coagulation, therapy-induced anticoagulation, and formation of new fibrin all illustrate the complexity of effects of the treatment and challenges the hemostatic balance in the patients. The therapy-induced effects can be modulated by parallel administration of anticoagulants. Risk assessment is mandatory prior to thrombolytic therapy. Anticoagulated and unconscious patients represent particular safety concerns, and should be fully evaluated. Several guidelines describe the choice of tests and their safety limits in relation to pretreatment evaluation of anticoagulated patients. Fibrinogen depletion and FDPs during treatment may be promising markers for the evaluation of bleeding risk posttreatment. Future risk assessment measures should focus on the dynamics of the hemostatic balance. Here, thromboelastography may be considered a tool addressing clot formation, fibrin structure, and fibrinolytic resistance in parallel. Suitable laboratory analysis performed shortly after treatment may help to recognize severe treatment-induced systemic effects that can be counteracted by rational treatment, thereby reducing bleeding risk.

Keywords

- thrombolysis
- systemic effects
- bleeding risk

Thrombolytic treatment with plasmin or plasminogen activators is meant to lyse thrombi and to recanalize a blood vessel or catheter. The target thrombus can emerge in different locations such as the deep veins of the leg (leading to deep venous thrombosis), in the lung artery (pulmonary embolism), and in arteries in the heart, brain (stroke), or leg, and can block bypass grafts or dialysis catheters.

The target thrombi can be of different composition and age, as documented in samples obtained by thrombectomy. With myocardial infarction (MI), it has been reported that nearly 50% of thrombi are older than 24 hours,¹ and it has been described that 9% of thrombi are older than 5 days.² This implies a quite wide spectrum in the composition of thrombi and a reduced accessibility when reendothelialization has

progressed in the presence of matured thrombi. It is now evident that acute myocardial infarction (AMI) refers to the acute clinical symptoms and not necessarily to completely acutely formed fresh thrombi.

In addition, the obstruction is not always a fully fibrin-based clot. The recognition of extracellular leukocyte networks with nucleotide strands in fibrin clots implies the need of DNA-ase to contribute to the lysis of such networks.^{3–5}

These refinements in knowledge of the target thrombus, mainly established for AMI, might provide clues for the observed apparent ceiling in recanalization rate (around 80–90%) that can be achieved by lysis strategies⁶ and count in favor of mechanical methods which can achieve higher patency rates.

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Related to the above-mentioned insights, in more recent years the single biological treatment with the thrombolytics for AMI has become less used and has been replaced to a large extent by mechanical methods of percutaneous coronary intervention (PCI), aspiration of thrombi, and combinations of lysis and mechanical strategies.

The present review concerns the safety associated with thrombolytics, used either alone or as treatment component. We focus on AMI and stroke, but all thrombolytic treatments have in common a depletion of internal hemostatic factors (systemic effects), anticoagulation, and a risk of bleeding associated with that systemic effect.

From evaluation of mechanisms and large interindividual variability in treatment and systemic response, it is suggested to select laboratory methods for the monitoring of systemic effects and the consequences in hemostasis for individuals to predict bleeding and importantly to rationally assist in management of bleeding, which irrevocably will affect a subset of patients undergoing thrombolytic treatment.

Mechanisms of Thrombolytics

Thrombolytics that have frequently been studied comprise alteplase (recombinant tissue-type plasminogen activator [rt-PA]), urokinase (urinary-type plasminogen activator, both in active and proform), reteplase (which is part of tissue-type plasminogen activator [t-PA], K2P, with reduced clearance rate), and tenecteplase (a genetically modified t-PA, possessing improved fibrin specificity and reduced inhibition by plasminogen activator inhibitor).

It has been well recognized that rapid lysis of thrombi by exogenously provided thrombolytics is a forced situation to achieve rapid lysis. The dosages of thrombolytics used are therefore very high compared with plasma levels of endogenous plasminogen activators. One of the premises for the high dose is that plasminogen activators incorporated into the thrombus during thrombus formation are far more effective than plasminogen activators deployed after thrombus formation. Furthermore, a t-PA dose in the high range has been selected in practice to be effective also with respect to older and organized clots.

The difference in concentration of lytic agents for endogenous and exogenous lysis has been illustrated by in vitro experiments showing that a 20 to 40 times higher concentration of t-PA is needed for exogenous lysis compared with endogenous lysis.^{7,8} Accordingly, levels of t-PA during thrombolysis reach 1 to 2 µg/mL, while endogenous free active t-PA in resting condition is around 1 ng/mL⁹ and reaches 10 ng/mL extra t-PA in plasma after exhaustive exercise,¹⁰ while locally endothelial stimulation can deliver 2 to 4 ng/min × L tissue to be incorporated in forming thrombi.¹¹

Mechanisms of Systemic Effects

Systemic effects have various origins as depicted in ▶Fig. 1,¹² and will be discussed here.

Systemic effects are mainly an issue of thrombolytic treatment due to the very high dosages used, as discussed earlier, where the fibrin specificity is not maintained.

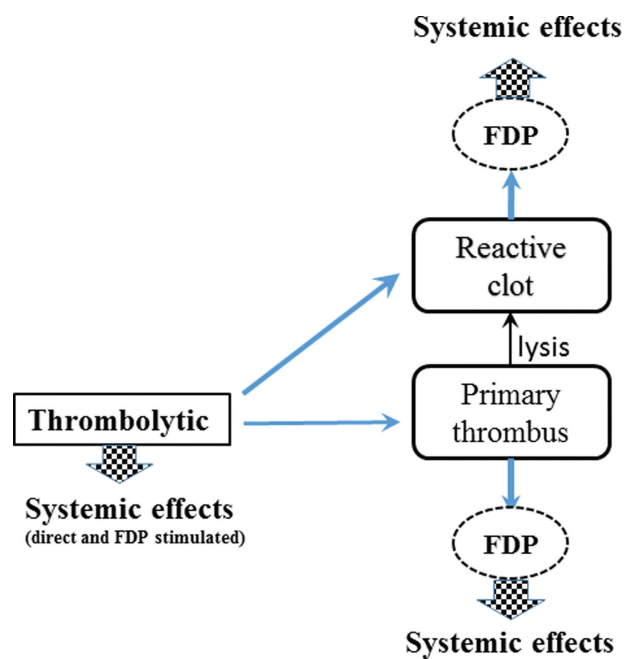


Fig. 1 Scheme of systemic effect origins. Systemic effects can be due to effects of thrombolytics without fibrin, effects stimulated by fibrin degradation products (FDPs) from the primary thrombus, and from clots/fibrin originating from reactive coagulation following thrombolysis.¹²

History documents that the thrombolysis originally performed by predominantly streptokinase therapy was accompanied by adverse effects, involving severe systemic reductions of fibrinogen, plasminogen, and plasmin inhibitor.¹³ The developments of newer thrombolytics were largely focused on improving fibrin specificity and reducing systemic effects. The thrombolytic agent evaluated extensively after streptokinase and urokinase was t-PA, and the reduced systemic effects of t-PA have been convincingly documented in the first head-to-head comparisons of streptokinase and t-PA.¹³ Treatment with t-PA induces less proteolysis of fibrinogen, factor VIII, and factor V than induced by streptokinase.

Several aspects of the mechanisms of systemic effects can be recognized. Plasminogen activation without fibrin is a well-known phenomenon for streptokinase, but also for urokinase and prourokinase,^{14,15} while it is less so for t-PA. The mutated t-PAs (reteplase and tenecteplase) show further reduction in systemic effects. The bat vampire plasminogen activator is reported to be the most favorable in this respect.¹⁶ These effects are studied with purified thrombolytics and components.^{17,18} In a plasma system, the presence of stimulating factors such as fibrin degradation products (FDPs) interferes with conclusions about mechanisms.

Next to the specific characteristics of a thrombolytic, the fibrin-independent effect is also strongly dependent upon dosages of the thrombolytics. Anno 2016, the systemic effect with current treatments is still too large and undesired and more fibrin-specific agents remain desired.^{19,20}

Fibrin Degradation Products' Effects

The concentrations of FDPs and large soluble fibrin polymers are elevated in patients with acute thrombotic diseases. However, already in normal individuals these factors are present and constitute soluble stimulating factors for plasminogen activation by t-PA. This was elegantly demonstrated by a chromogenic assay based on stimulation of t-PA activity showing in normal volunteers a level of $\sim 3 \mu\text{g}$ fibrinogen/fibrin equivalents/mL of "soluble fibrin."²¹ During thrombolytic treatment, these levels can increase 5- to 10-fold and are supposed to not represent the small primary thrombus as main source.²² This latter assumption is based on calculations of the size of thrombi and expected amounts of FDPs and on experiments showing generation of FDPs upon incubating plasma with a lytic agent, and provided evidence for "new" FDP generated by an ongoing coagulation processes during thrombolytic treatment.²² The stimulation is predominantly from fragments containing D and E fragments (DDE),²³ which are normally included in so-called D-dimer assays and in assays employing the E-domain for catching or tagging.^{24,25}

Major Effects and a Threshold

Very large off-target effects of plasmin can occur, and are due to the situation that the inhibitory capacity for plasmin in plasma is not sufficient to achieve a rapid neutralization of all plasmin that can be formed. The level of plasminogen is around $2 \mu\text{M}$ in normal human plasma, while the level of rapidly acting plasmin inhibitor (the plasminogen-binding form) is only $0.7 \mu\text{M}$.²⁶ This rapid plasmin inhibitor exerts one of the fastest biological interactions addressing non-fibrin-bound plasmin.²⁷ After this very effective inhibitory barrier, the inhibition by the non-plasminogen-binding plasmin inhibitor ($0.3 \mu\text{M}$) and by α -2-macroglobulin ($2 \mu\text{M}$) is slower. The slower inhibition allows plasmin, as broad-spectrum protease, to also proteolyze other proteins more effectively. In hemostasis, the well-known "victims" after fibrinogen are von Willebrand factor,²⁸ factor VIII,^{29,30} and factor V and Va,³¹⁻³³ all shown to be sensitive to plasmin. Also factor IX and factor X are plasmin sensitive,^{34,35} and to a minor extent factor XIII.³⁶⁻³⁸ The formation of large fibrin and fibrinogen degradation products with the capacity to stimulate in solution t-PA-induced plasminogen activation occurs predominantly after passing the highly effective inhibitory barrier of plasminogen-binding plasmin inhibitor and can be counteracted by providing additional plasmin inhibitor.³⁹

It may be clear that degradation of clotting factors: (1) impairs coagulation, (2) that formed fibrin and fibrinogen degradation products that impair fibrin polymerization and stimulate plasmin formation in solution, and (3) that the exhaustion of fibrinolysis inhibitors facilitate lysis of clots. This renders systemic effects an important determinant of the risk of bleeding. It is of importance to recognize that systemic effects are quite different among patients. Residual fibrinogen analysis illustrates this aspect clearly for t-PA treatment (see **Fig. 2**¹³), demonstrating residual fibrinogen concentrations in a very broad range.

It can be concluded that one important issue regarding selection of improved thrombolytics is related to reduction of systemic effects. In addition, variable susceptibility among

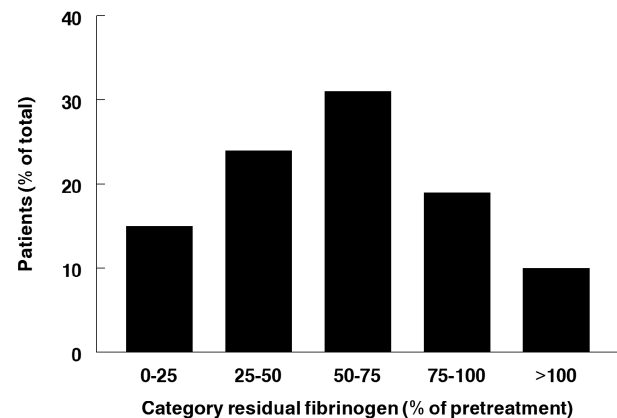


Fig. 2 Categories of patients with different residual fibrinogen after t-PA treatment. Data taken from Collen et al.¹³

individuals may call for laboratory diagnostic testing to both recognize susceptible patients and identify the actual consequences of systemic adverse effects.

Evaluation of the Ratio between Thrombolysis and Systemic Effects

In early phases of development of a new thrombolytic, its dose-dependent efficacy and side effects can be documented in detail with specialized laboratory methods. This has been done in the past for agents including streptokinase, APSAC (anisoylated plasminogen streptokinase activator complex), t-PA, urokinase prourokinase, reteplase, tenecteplase, desmoteplase, and (micro)plasmin, and is still ongoing for new agents and treatment modulation. Such a new agent is a mutant of pro-u-PA which upon activation is specifically more strongly inhibited by C1-inactivator, reducing its non-fibrin impact⁴⁰. A treatment modality that mimics endogenous synergy between t-PA and prourokinase using combination treatment is also an option, although it is less well explored, to increase fibrin specificity.¹⁹

In Vitro Dose Finding

In vitro methods for efficacy concern typically clot lysis tests by an exogenously provided thrombolytic, involving, for instance, hanging clot and Chandler loop principles.^{41,42} The methods for evaluation of side effects can be used in a combination of the same tests by evaluation of non-clot-bound actions in the surrounding blood or plasma. This can also be done by incubation of plasma with the thrombolytic agent. Such tests can include assays of plasminogen consumption, residual rapid plasmin inhibitor, formation of plasmin-plasmin inhibitor complexes, plasmin- α -2-macroglobulin complexes, and formation of FDPs, and proteolysis of fibrinogen to its B β 1-42 fragments and degradation/inactivation of factor VIII, factor V, and von Willebrand factor. An interesting option arises from the availability of an assay specific to fibrinogen degradation products reporting off-target proteolytic action of plasmin on fibrinogen.²⁴

The in vitro ratio fibrin specificity/efficacy and side effects (systemic effects) can be documented for different dosages

and benchmarked with rt-PA. It can be used to support the selection of dosages for treatment and positioning of the new thrombolytic agent relative to existing ones.

In dose-finding studies in individuals, the same assays can be applied for the systemic effects (provided the sample is stabilized when necessary by addition of inhibitors). It should be noted, as discussed earlier, that laboratory studies on FDPs for analysis of the lysis of pathological thrombi (unless massive) are not options for efficacy in patient treatment (see discussion later). Here, recanalization and eventually survival and risk of reinfarction are the relevant end points.

Dynamics of Lysis and Coagulation in the Patients during Thrombolysis

Treatment-Induced New Fibrin Formation

Lysis of an occluding thrombus is expected to result in reperfusion and in uncovering thrombogenic surfaces, and potentially the thrombolytic is involved in removing hemostatic fibrin clots or counteracting new formation of hemostatic clots. Activation of the coagulation mechanism not only is plausible, but also has been documented to occur during thrombolysis (see the discussion later). Accordingly, *in vivo*, the lysis of the thrombus with a thrombolytic is not a linear process of lysis, but involves also a feedback activation of coagulation and formation of new clots. Thus, lysis has to cope with existing and newly formed fibrin due to the treatment. This new coagulation effect can contribute to reocclusion and eventually reduced efficacy of the treatment. This dynamic process of treatment-induced coagulation is well recognized in MI (also coined as thrombolytic paradox) and is documented in animal models where experimental clots show lysis and growth.⁴³ Treatment-induced coagulation is also documented in MI patients by the generation of coagulation activation products such as prothrombin fragment 1 + 2 (thrombin generation marker), thrombin-antithrombin com-

plex (thrombin presence marker), and fibrinopeptide A (thrombin action marker). A consequence is that the concentration of FDPs is much higher than can be expected from the existing thrombus due to substantial contribution from the additional lysis of the newly formed fibrin. The treatment-induced coagulation and the prothrombotic condition of the infarcted area, in combination with the apparent prothrombotic phenotype of the infarct patient, can result in reocclusion of the target vessel. The coagulation process involved can be modulated by the use of inhibitors such as heparins and platelet inhibitors, which are carefully selected and evaluated for effect and steady companions in the thrombolytic treatment of MI and under investigation for stroke, and still subject of research to improve effects.⁴⁴ The treatment-induced coagulation is related to the specific thrombolytic, the dosage, and way of administration. As an example, this is illustrated by differences in generated activation markers of coagulation when comparing infusion and bolus (→ Fig. 3).^{45,46}

Modulation of Treatment-Induced Coagulation

Treatment-induced coagulation can be modulated by inhibition of coagulation in parallel with the administration of the thrombolytic agent. Initially, heparin and/or antiplatelet agents were used as modulators in patients with MI. Current recommendations include adjuvant regimens with enoxaparin, unfractionated heparin (UFH), and fondaparinux.⁴⁷ Evaluation of rt-PA combined with platelet glycoprotein IIB-IIIa inhibitors is still under study for stroke.^{48,49}

More recent approaches also include the possibilities of the inhibition of contact activation, factor XI action, thrombin inhibition, and TAFI (thrombin-activatable fibrinolysis inhibitor) inhibition. It has been recognized that thrombus stabilization follows activation of the intrinsic system (factor XII, prekallikrein, and in particular factor XI), while the thrombin formed by this route activates TAFI, which is an inhibitor of fibrinolysis and retards clot lysis.⁵⁰ This is expected to have

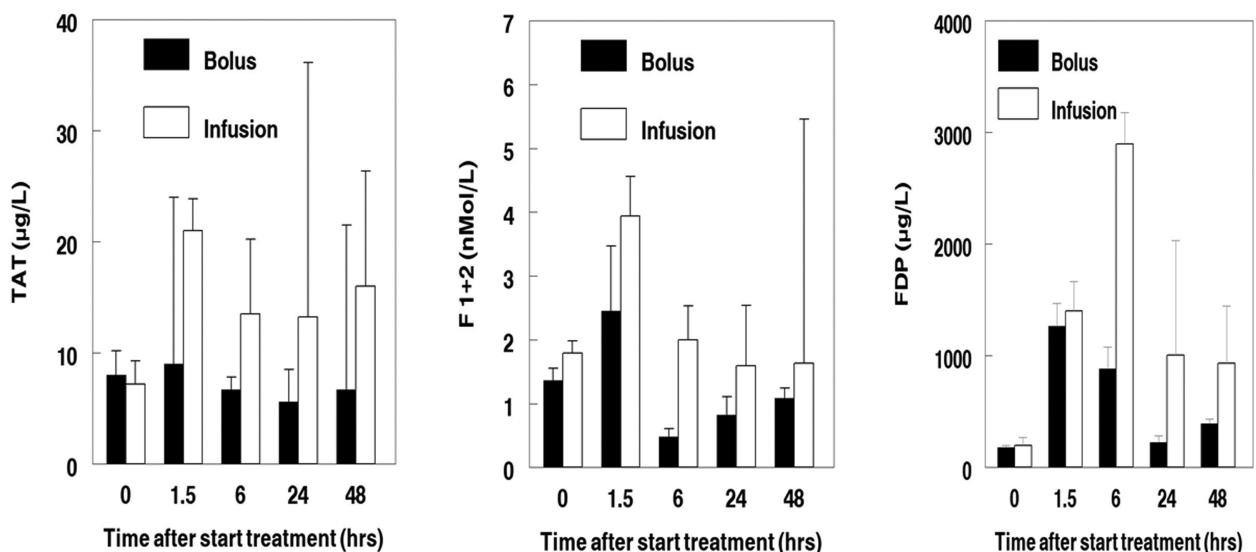


Fig. 3 Molecular markers during and after treatment with bolus t-PA (four boluses in 1 hour) and infusion of t-PA for a total of 6.5 hours for patients showing recanalization. Data are from the study of Andreotti et al,⁴⁶ and medians and standard errors are plotted.

contributed to stabilization of the culprit thrombus in infarct-related areas and is operational indeed for the initial thrombus, as suggested by increases in factor XIa–C1-inactivator complex⁵¹ and by the thrombotic effects in experimental studies in factor XII- or prekallikrein-depleted mice, in case of inhibitors of factor XIIIa (mice and rat model),⁵² in case of inhibition of factor XI in mice and rabbit models,⁵³ and by XIIIa and XIa inhibition in extracorporeal circulation and grafts.^{54,55}

Specific options for modulation of treatment-induced coagulation may be targeted at TAFI because of positive reports showing that, in TAFI-deficient mice, venous thrombi are smaller and TAFI polymorphism influences stroke burden.⁵⁶ TAFI inhibition shows less microthrombosis⁵⁷ and fibrin deposition in the lung in experimental models,⁵⁸ and it induces more lysis in a rabbit model⁵¹ and potentiates jugular vein lysis.⁵⁹

The occurrence of this mechanism of thrombus stabilization via the intrinsic route is likely to operate also in the new thrombolytic-induced coagulation. Some experimental evidence is provided from increases during thrombolysis in activated factor XII^{60,61} and in cleaved high-molecular-weight kininogen.^{60,62,63} Interestingly long-lasting depletion of the FXII-dependent pathway of fibrinolysis has been reported in patients undergoing thrombolytic therapy with rt-PA, thereby carrying an increased risk of early reinfarction.⁶⁴ More thorough documentation is warranted, and based on such observations regarding the role of the contact system and TAFI, new adjuvant treatment may arise in the near future.⁶⁵

A focused way to modulate treatment-induced coagulation could be by inhibition of TAFI formation or TAFI directly.^{66,67} TAFI participation requires thrombomodulin, which will be a major co-determinant. Inhibition of thrombin and subsequent TAFI formation by the use of argatroban indeed stimulates lysis.⁶⁸

Systemic Effects during Treatment

Of importance is that the systemic effects are stimulated by lysis of the thrombus, which results in the formation of large FDPs that show stimulatory action for t-PA.²³ Paradoxically, the larger the effects of the fibrinolysis target (existing + newly formed thrombi), the larger the systemic effects may become.

The systemic effects reduce the quality of newly formed fibrin clots by several mechanisms:

- The amount of fibrinogen is reduced, which may result in a smaller clot.
- Fibrin and fibrinogen degradation products disturb fibrin polymerization and cause a change in fibrin structure.⁶⁹
- Reduced coagulation results in impaired cross-linking (factor XIII) and reduced inhibitor formation (TAFI activation).

In addition, the anticoagulant used to prevent reactive coagulation during and after treatment adds to impairment of coagulation activation and consequently the risk of bleeding.

The complex situation in vivo during thrombolysis and the application of different thrombolytics and regimen with anticoagulation lead to a set of rules about systemic effects.

Reduced systemic effects link with:

- Inhibition of rethrombosis and reducing treatment-induced clot formation
- Fibrin specificity of the lytic agent as more fibrin specificity causes less systemic effect by fibrin-independent effects, by low circulating soluble fibrin and by low large fibrin (ogen) breakdown products

Increased systemic effects link with:

- Larger thrombi and a strong prothrombotic phenotype of the patient
- Higher dosage of a thrombolytic or prolonged infusion schemes
- Coagulation inhibition targeting at the intrinsic pathway, which gives more/faster lysis

Thus, treatment with thrombolytics requires strategies reducing the treatment-induced activation of coagulation, thereby further decreasing the bleeding risk. For assessment of bleeding risk, two lines could be followed: (1) evaluation of the patient before treatment for risk factors for bleeding and (2) evaluation of the patient after completion of treatment for risk of posttreatment bleeding.

Identification of Patients for Thrombolysis: Risk Assessment

Major bleeding is still a significant problem of thrombolytic therapy, despite the development of fibrin-specific drugs. For all thrombolytics available today, there are to a certain extent systemic adverse effects of which hemorrhagic stroke is considered the most feared complication. Thrombolytic agents that are presently available do not have the capability to distinguish between pathological thrombi and normal hemostatic fibrin plugs. The risk of hemorrhagic stroke ranges from 1 to 7% depending on the target organ of the thrombolytic agent.^{70–72} The current scope for use of intravenous thrombolytic agents is thrombotic stroke, STEMI (ST segment elevation myocardial infarction), pulmonary embolism, and to a lesser extent vein thrombosis. Because of the high treatment-induced bleeding risk, major efforts should be undertaken to exclude patients who have a higher risk for bleeding. It should be noted that only alteplase is FDA approved for thrombolysis of thrombotic stroke. Also, it should be noted that the primary treatment of STEMI is PCI and that thrombolytic treatment of non-STEMI is not indicated.

The efforts to reduce the bleeding risk in thrombolytic therapy have resulted in recommendations on absolute contraindications. Such recommendations are based on clinical information or biochemical measures. ►Table 1⁷³ summarizes the hemostasis contraindications.

Also, it is recommended to perform biochemical tests to help guide whether the patients are eligible for thrombolytic therapy (►Table 2⁷³).

Table 1 Clinical information to decide eligibility of patients for thrombolytic therapy

- Time from stroke symptoms ≥ 4.5 h
- Time from MI symptoms ≥ 24 h
- Previous intracranial hemorrhage or stroke of unknown origin
- Blood pressure $> 185/110$ mm Hg after two attempts to reduce blood pressure
- Active gastrointestinal bleeding within 1 mo
- Surgery and trauma within 14 d
- Noncompressible punctures within 24 h (liver biopsy, etc.)
- Ischemic nerve system/neoplasms/known atrioventricular malformation
- Current use of warfarin INR > 1.7
- Current use of direct thrombin or factor Xa inhibitors (DOACs)

Abbreviations: DOACs, direct oral anticoagulants; INR, international normalized ratio; MI, myocardial infarction.
Source: Adapted from Jauch et al.⁷³

Laboratory Tests (Pretreatment)

Identification of patients at risk of bleeding prior to thrombolytic therapy is mandatory to ensure the safety of the treatment. Patients subjected to thrombolytic therapy mostly have a prothrombotic phenotype and will not generally suffer from inherited bleeding tendency, that is, coagulation factor deficiencies and platelet disorders. The majority of patients, however, may have an acquired bleeding risk due to anticoagulant treatment, that is, heparins, vitamin K antagonists (VKAs), and direct oral anticoagulants (DOACs; being direct thrombin inhibitors [dabigatran, argatroban] and direct or indirect inhibitors of FXa [rivaroxaban, apixaban, fondaparinux]). Particular problems are related to unconscious patients who are possibly on treatment with DOACs, as tests to exclude use of DOACs are not widely available in routine laboratories. However, to ensure the safety of the treatment, the laboratory analysis repertoire should identify these conditions before thrombolytic therapy is initiated.

According to international guidelines, global tests such as the prothrombin time (PT) and the activated partial prothrombin time (aPTT) may be particularly useful for identification of patients with increased risk of bleeding, while ruling out patients receiving low-molecular-weight (LMW) heparin

Table 2 Biochemical measures to guide eligibility of patients for thrombolytic therapy

Blood glucose
Oxygen saturation
Electrolytes/EGFR/creatinine
Complete blood count, including platelets
Prothrombin time (PT)
Activated partial thromboplastin time (aPTT)
Electrocardiography (ECG)
Troponins

Abbreviation: EGFR, estimated glomerular filtration rate.
Source: adapted from Jauch et al.⁷³

and to some extent DOACs that require more specific tests.^{73–75} Quick access to relevant laboratory results is important for the visitation of patients for and on thrombolytic therapy. Hence, point-of-care test (POCT) devices are advantageous for laboratory testing in the thrombolytic setting, because the turnaround time of the tests is short and the analysis can be performed at bedside. Guidelines focusing on the preanalytical and analytical conditions of the assay procedures should be followed to ensure that the outcome of the analytical work is reliable.^{75–77}

Testing for Vitamin K Antagonists

The test of choice ruling out VKA treatment is the PT addressing the coagulation capacity of the tissue factor-induced (extrinsic) and common coagulation pathway. A variety of PT assays and applications are available, and the PT can be reliably performed using POCT devices.^{78,79} The outcome of the analysis can be expressed in seconds, ratio, and international normalized ratio (INR).⁸⁰ Ischemic stroke patients on VKA treatment with INR < 1.7 can safely be treated with intravenous rt-PA.^{73,81,82} PT values below 15 seconds are also considered safe,⁷³ but it should be noted that the clotting time of the PT depends on the assay conditions and the equipment used for analysis. Thus, a PT safety limit expressed in seconds is not recommendable.

Testing for Heparins

The aPTT addresses the coagulation capacity of the surface-induced (intrinsic) and common coagulation pathway. The aPTT is sensitive to UFH. Only few POCT devices capable of measuring aPTT are presently available^{83–85} and their performance in a thrombolytic setting has not been evaluated so far. The outcome of the aPTT can be expressed in seconds or ratio. An aPTT ratio within the reference range of the assay prior to thrombolysis is considered as safe,⁷³ whereas other guidelines recommend a safety range of the aPTT ratio as ≤ 1.5 times the baseline value.⁷⁴ It is, however, not clear how this baseline value is defined. It should be noted that the aPTT is not suitable for detection of LMW heparin or fondaparinux, which should be determined with anti-FXa assays.⁷⁵

Testing for Direct Oral Anticoagulants

Treatment with direct thrombin or FXa inhibitors may prolong the PT and aPTT, but these measurements are not reliable for measuring the pharmacokinetic or pharmacodynamic effects of DOACs. The aPTT, however, may be used to screen for presence of dabigatran and rivaroxaban, but the aPTT is unsuitable to determine the concentration of the drugs. A normal aPTT cannot exclude the presence of dabigatran in some patients, and both the PT and aPTT are insensitive to apixaban.

The thrombin time (TT) and ecarin clotting time (ECT) are both sensitive to the presence of direct thrombin inhibitors, and normal clotting times with these tests suggest very low plasma levels of these drugs. Studies have suggested using the combination of TT and aPTT measurements as a safety indicator prior to thrombolysis.⁸⁶ It is, however, recommended that dilute thrombin-based assays, ECT-based assays, or chromogenic anti-IIa assays should be used for determination of

dabigatran, whereas chromogenic anti-Xa assay should be used for determination of direct Xa inhibitors such as rivaroxaban. A variety of specific chromogenic anti-IIa and anti-Xa assays are available for measurement of DOACs,^{83,87-92} and several of these assays are available as POCT. It is of particular note that all assays should be calibrated with drug-specific calibrators. A thorough and comprehensive guideline describing the measurement of DOACs has been published recently.⁷⁵

Recent guidelines state that patients treated with DOACs should not be subjected to thrombolytic therapy with rt-PA unless the aPTT, PT, and platelet count are normal. Moreover, safe treatment with rt-PA requires that the ECT, TT, or appropriate direct FXa activity assays are normal, or that the patient has not received a dose of DOACs for >2 days.⁷³ This recommendation, however, is rather weak (class III; level of evidence C) and further research is needed to consolidate the safety limits of DOACs. The recommended tests and safety limits in anticoagulated patients are listed in **Table 3**.

Thromboelastography (TEG) and rotational thromboelastometry (ROTEM) are promising tools to investigate the anticoagulant effect of DOAC as demonstrated by in vitro studies,⁹³ and the techniques can also be used for detection of factor deficiencies, presence of heparins, and VKAs. Clinical trials focusing on apixaban and rivaroxaban have shown that TEG may be a suitable tool for the determination of DOACs, also in the thrombolytic setting,^{94,95} but the precision of the assays in some cases is poor.⁹⁶ More studies are needed to reach firm conclusions regarding the usage of TEG and ROTEM in relation to thrombolytic risk assessment.

Laboratory Tests (Posttreatment)

Reduction in the plasma concentration of fibrinogen after thrombolytic therapy is significantly associated with risk of posttreatment bleeding.⁹⁷⁻¹⁰⁰ A large clinical trial with more than 500 patients receiving rt-PA as thrombolytic agent demonstrated that a reduction in fibrinogen of ≥ 2 g/L from baseline to 6 hours after therapy increased the risk of bleeding in the first 72 hours after therapy more than four

times, and that the negative predictive value of fibrinogen depletion for any major bleeding was 94%.⁹⁷ Another study, assessing the fibrinogen concentration before and 2 hours after rt-PA therapy, showed that a reduction in fibrinogen concentration of 25% or more during therapy, or a fibrinogen concentration ≤ 2 g/L after therapy increased the bleeding risk in the first week after therapy more than seven times.⁹⁸

For comparison, a study of patients subjected to rt-PA treatment due to peripheral arterial or venous thrombosis showed that the rate of major bleeding was significantly higher for patients with a fibrinogen level ≤ 1.5 g/L.⁹⁹ These studies demonstrate that plasma fibrinogen levels may serve as a posttreatment safety indicator of thrombolytic therapy.

Coinciding with a nadir in fibrinogen, treatment of stroke or MI with rt-PA showed a peak in FDPs between 1 and 4 hours.^{37,101} Sampling at 2 hours after start of treatment showed a relationship of FDP level with hemorrhage in several studies.¹⁰¹⁻¹⁰⁴ High FDPs at 24 hours was suggested as contraindication for antithrombotic drugs in the first 72 hours after stroke.¹⁰² These studies demonstrate that FDPs levels may also serve as a posttreatment safety indicator of thrombolytic therapy. It is suggested to further invest in comparisons of FDP assays in view of the data (see later) that DDE containing fragments are more active in systemic activation.

It should be noted that anticoagulant therapy, irrespective of the drug used, should not be initiated in stroke patients within 24 hours of treatment with intravenous rt-PA.⁷³

Conclusion and Future Options

The bleeding diathesis in thrombolysis treatment shows similarities with bleeding diathesis in traumatic and post-surgery situations. ROTEM or TEG analyses have been used in these settings, and may also be promising tools for evaluation of the coagulation status prior and after thrombolytic therapy.

Table 3 Recommendations of choice of tests and safety limits in patients receiving anticoagulant treatment prior to thrombolytic therapy

Drug	Recommended test	Safety limit
Vitamin K antagonists	Prothrombin time	INR < 1.7 PT < 15 s
Unfractionated heparin	Activated partial thromboplastin time	Values within reference range aPTT < 1.5 \times baseline
LMW heparin	Anti-Xa assay	Not applied
Fondaparinux	Anti-Xa assay	Not applied
Direct thrombin inhibitors Dabigatran Argatroban	Thrombin time Ecarin clotting time Chromogenic anti-IIa assay	Values within reference ranges Last dosage > 2 d ago
Direct factor Xa inhibitors Rivaroxaban Apixaban	Chromogenic anti-Xa assay	Values within reference ranges Last dosage > 2 d ago

Abbreviations: aPTT, activated partial thromboplastin time; INR, international normalized ratio; LMW, low molecular weight; PT, prothrombin time.

These analyses, performed at bedside and using whole blood, show sensitivity toward most coagulation disorders, platelet dysfunction, and presence of various anticoagulants. We have previously demonstrated that ROTEM analysis prior to surgery is predictive of intraoperative bleeding in an orthognathic setting.¹⁰⁵

Evaluation of fibrinogen turnover during thrombolytic therapy may be another option ensuring the safety of treatment. Patients depleted in fibrinogen during surgery and identified with ROTEM or TEG analyses could be supplemented with fibrinogen to prevent postsurgical bleeding, according to the most recent guidelines from the European Society of Anaesthesiology.¹⁰⁶ In contrast, a Cochrane Database Systemic Review concludes that currently only weak evidence supports the use of fibrinogen concentrate in bleeding patients, tested in primarily elective cardiac surgery, and that more research is urgently needed.¹⁰⁷

A further advancement may be the application after thrombolysis of a method incorporating data on fibrin quality and lysis susceptibility. A complex phenotypic test reflecting all elements of risk of bleeding in thrombolysis patients—reduced clotting, reduced elasticity, increased sensitivity to lysis programmed by low thrombin formation, and reduced TAFI activation—and the interrelations might be a rational, theoretical choice. To that end, it is possible to select a ROTEM variant in line with those proposed,¹⁰⁸ with low clotting stimulation (low tissue factor) to give way to expression of the endogenous clotting status and to express effects of TAFI, and to add t-PA to the test. It is suggested to explore this type of approach and perform studies to evaluate its validity to possibly reach high efficacy in risk analysis.

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